

I claim:

1. A method of constructing a product polynucleotide using populations of truncate-containing oligonucleotide synthesis products, the method comprising:

partially duplexing a scaffold oligonucleotide of subtemplate length having a first terminus and a second terminus with a central oligonucleotide that has a 5' terminus and a 3' terminus, such that a single-stranded region is left at both the first and second termini of the scaffold oligonucleotide; and

ligating a first and a second oligonucleotide, respectively, to the 5' and 3' termini of said central oligonucleotide by sampling respective first and second populations of truncate-containing oligonucleotide synthesis products with the single-stranded regions at the termini of said partially duplexed scaffold oligonucleotide,

said sampling being performed in the presence of a ligase under conditions in which hybridization of said first and second oligonucleotides to the single-stranded regions at the termini of said scaffold oligonucleotide is unstable, and in which hybridization of said central oligonucleotide to said scaffold oligonucleotide is stable,

wherein the first oligonucleotide includes a region perfectly complementary in sequence to the single-stranded region at the first terminus of said scaffold oligonucleotide and the second oligonucleotide

includes a region perfectly complementary in sequence to the single-stranded region at the second terminus of said scaffold oligonucleotide.

2. The method of claim 1, wherein said partial duplexing and said ligating are performed in a single step.

3. The method of claim 1, further comprising a step of size separating the product polynucleotide from the scaffold oligonucleotide.

4. The method of claim 1, wherein each of the single-stranded regions at the termini of the scaffold oligonucleotide is no more than 10 nucleotides long.

5. The method of claim 1, wherein each of the single-stranded regions at the termini of the scaffold oligonucleotide is no more than 7 nucleotides long.

6. The method of claim 1, wherein each of the single-stranded regions at the termini of the scaffold oligonucleotide is no more than 5 nucleotides long.

7. The method of claim 1, wherein the conditions of the ligating step include a temperature of at least 30°C.

8. The method of claim 1, wherein the conditions of the ligating step include a temperature of at least 42°C.

9. The method of claim 1, wherein the conditions of the ligating step include a temperature of at least 50°C.

10. The method of claim 1, wherein the product polynucleotide extends at least 10 nucleotides beyond each of the termini of the scaffold oligonucleotide.

11. The method of claim 1, wherein the product polynucleotide extends at least 25 nucleotides beyond each of the termini of the scaffold oligonucleotide.

12. The method of claim 1, wherein the product polynucleotide extends at least 75 nucleotides beyond each of the termini of the scaffold oligonucleotide.

13. The method of claim 1, wherein said first sampled population includes the products of a first plurality of oligonucleotide syntheses, the full-length synthesis products of at least two of said first plurality of syntheses being different in sequence, and said second population includes the products of a second plurality of oligonucleotide syntheses, the full-length synthesis products of at least two of said second plurality of syntheses being different in sequence.

14. The method of claim 1, wherein the scaffold oligonucleotide is partially duplexed to the central oligonucleotide, such that a single-stranded region is left at both the first and second termini of the scaffold oligonucleotide, prior to contacting the scaffold and central oligonucleotides with the first and second oligonucleotides.

15. A method of constructing at least one species of product polynucleotide using populations of truncate-containing oligonucleotide synthesis products, the method comprising:

ligating together a center oligonucleotide, at least one species of first oligonucleotide, and at least one species of second oligonucleotide,

the first, center and second oligonucleotides being annealed to a common scaffold oligonucleotide during ligation,

the scaffold oligonucleotide being complementary to the entire length of the center oligonucleotide, such that the duplex formed is stable under ligation conditions,

the scaffold oligonucleotide being complementary to the first and second oligonucleotides only over a limited number of nucleotides, such that the duplexes formed are not stable under ligation conditions,

and wherein the scaffold oligonucleotide does not provide complementary nucleotides along substantially the full length of the first oligonucleotide, and does not provide complementary nucleotides along substantially the full length of the second oligonucleotide.

16. A method of appending at least one species of extending oligonucleotide to an end of a single-stranded polynucleotide, comprising:

combining the extending oligonucleotide and the single-stranded polynucleotide with a scaffold oligonucleotide, said scaffold oligonucleotide being complementary along a portion of its length to the single-stranded polynucleotide, and said scaffold oligonucleotide being complementary along another portion of its length to the extending oligonucleotide, such that the single-stranded polynucleotide and the extending oligonucleotide are properly aligned for ligation when both are base paired to the scaffold oligonucleotide; and

ligating the extending oligonucleotide to the single-stranded polynucleotide.

17. The method of claim 16, wherein the ligation step is performed under solution conditions in which the scaffold oligonucleotide forms an unstable duplex with the single-stranded polynucleotide, and in which the scaffold oligonucleotide forms a stable duplex with the extending oligonucleotide.

18. The method of claim 16, wherein the ligation step is performed under solution conditions in which the scaffold oligonucleotide forms a stable duplex with the single-stranded polynucleotide, and in which the scaffold oligonucleotide forms an unstable duplex with the extending oligonucleotide.

19. The method of claim 16, wherein the extending oligonucleotide comprises a primer binding site for use in polymerase chain reaction amplification.

20. The method of claim 16, wherein the extending oligonucleotide comprises a binding site for a type-IIS restriction endonuclease.

21. The method of claim 16, wherein the extending oligonucleotide comprises a bar code sequence.

22. The method of claim 16, wherein the extending oligonucleotide comprises a labeling oligonucleotide, said labeling oligonucleotide being detectable.

23. A method of appending at least one species of first extending oligonucleotide to the 5' end of a single-stranded polynucleotide, and appending at least one species of second extending oligonucleotide to the 3' end of the single-stranded polynucleotide, comprising:

combining the first extending oligonucleotide and the single-stranded polynucleotide with a first scaffold oligonucleotide, said first scaffold oligonucleotide being complementary along a portion of its length to the 5' terminal region of single-stranded polynucleotide, and said first scaffold oligonucleotide being complementary along another portion of its length to the first extending oligonucleotide, such that the single-stranded polynucleotide and the first extending oligonucleotide are properly aligned for ligation when both are base paired to the first scaffold oligonucleotide;

combining the second extending oligonucleotide and the single-stranded polynucleotide with a second scaffold oligonucleotide, said second scaffold oligonucleotide being complementary along a portion of its length to the 3' terminal region of single-stranded polynucleotide, and said second scaffold oligonucleotide being complementary along another portion of its length to the second extending oligonucleotide,

such that the single-stranded polynucleotide and the second extending oligonucleotide are properly aligned for ligation when both are base paired to the second scaffold oligonucleotide; and

ligating the first and second extending oligonucleotides to the single-stranded polynucleotide.

24. The method of claim 23, wherein the ligation step is performed under solution conditions in which the first and second scaffold oligonucleotides form unstable duplexes with the single-stranded polynucleotide, and in which the first and second scaffold oligonucleotides form stable duplexes with the first and second extending oligonucleotides, respectively.

25. The method of claim 23, wherein the ligation step is performed under solution conditions in which the first and second scaffold oligonucleotides form stable duplexes with the single-stranded polynucleotide, and in which the first and second scaffold oligonucleotides form unstable duplexes with the first and second extending oligonucleotides, respectively.

26. The method of claim 23, wherein the first and second extending oligonucleotides each comprise a primer binding site for use in polymerase chain reaction amplification.

27. The method of claim 26, wherein the primer binding site of the first extending oligonucleotide is not the same as the primer binding site of the second extending oligonucleotide.

28. The method of claim 23, wherein the first and second extending oligonucleotides each comprise a binding site for a type-IIS restriction endonuclease.

29. The method of claim 28, wherein the type-IIS restriction endonuclease binding site of the first extending oligonucleotide is not the same as the type-IIS restriction endonuclease binding site of the second extending oligonucleotide.

30. The method of claim 23, wherein the first and second extending oligonucleotides each comprise a bar code sequence.

31. The method of claim 30, wherein the bar code sequence of the first extending oligonucleotide is not the same as the bar code sequence of the second extending oligonucleotide.

32. A kit for labeling one or more oligonucleotides to be labeled, comprising:

at least one species of labeling oligonucleotide, said labeling oligonucleotide being detectable;

a labeling scaffold oligonucleotide, said labeling scaffold oligonucleotide being complementary along a portion of its length to the oligonucleotides to be labeled, and said labeling scaffold oligonucleotide being complementary along another portion of its length to the labeling oligonucleotide, such the oligonucleotides to be labeled and the labeling oligonucleotide are properly aligned for ligation when



both are base paired to the labeling scaffold oligonucleotide; and

instructions directing a user to;

combine the labeling oligonucleotide and the oligonucleotides to be labeled with the labeling scaffold oligonucleotide; and

ligate the labeling oligonucleotide to the oligonucleotides to be labeled under solution conditions in which the labeling scaffold oligonucleotide forms a stable duplex with the oligonucleotides to be labeled, and in which the labeling scaffold oligonucleotide forms an unstable duplex with the labeling oligonucleotide.

33. A kit for labeling one or more oligonucleotides to be labeled, comprising:

at least one species of labeling oligonucleotide, said labeling oligonucleotide being detectable;

a labeling scaffold oligonucleotide, said labeling scaffold oligonucleotide being complementary along a portion of its length to the oligonucleotides to be labeled, and said labeling scaffold oligonucleotide being complementary along another portion of its length to the labeling oligonucleotide, such the oligonucleotides to be labeled and the labeling oligonucleotide are properly aligned for ligation when both are base paired to the labeling scaffold oligonucleotide; and

instructions directing a user to;

combine the labeling oligonucleotide and the oligonucleotides to be labeled with the labeling scaffold oligonucleotide; and

ligate the labeling oligonucleotide to the oligonucleotides to be labeled under solution conditions in which the labeling scaffold oligonucleotide forms an unstable duplex with the oligonucleotides to be labeled, and in which the labeling scaffold oligonucleotide forms a stable duplex with the labeling oligonucleotide.

34. A method of ligating at least one species of first oligonucleotide to a second oligonucleotide, comprising:

combining the first oligonucleotide with the second oligonucleotide; and

ligating the first oligonucleotide to the second oligonucleotide under conditions in which the presence of a ten-fold molar excess of truncated forms of the first oligonucleotide inhibits the ligation of the first oligonucleotide to the second oligonucleotide by less than a factor of three.